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The Application of Plasmonic Nanostructures for Quantitative Measurements of Cell Secretions

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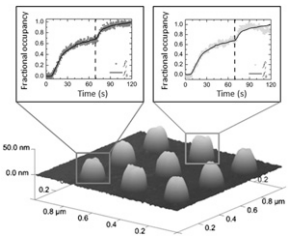
The localized surface plasmon resonance (LSPR) of lithographically patterned gold nanostructures was utilized for the quantitative measurement of anti-c-myc antibodies as harvested from a cultured 9E10 hybridoma cell line. The fact that the technique is label-free allowed for the antibody detection to be made without the need for further purification or processing. The simultaneous acquisition of CCD imagery and LSPR spectra enabled the calibration of hundreds of individual nanostructures in parallel. From the calibration we mapped the fractional occupancy of surface-bound receptors at individual nanostructures with nanomolar sensitivity and a temporal resolution of 225 ms. The setup is integrated on to an inverted microscope and fully compatible with other imaging techniques such as fluorescence and DIC.



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Development of Adaptive SEM Technology for Genome/Proteome Expression Analysis in Single Cell Level

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Identification of each cellular phenotype in a tissue is essential for understanding community effect in living system. One useful way is in situ measurement of expressed biomarkers in single cell level using a lot labels; however, production and identification of such labels are still challenging. We propose a new sensing technology, which is a comprehensive development of production and identification of nano-particle (NP) labels for simultaneous in situ measurements of expressed biomarkers in single cell.

For the fabrication of NPs, various sizes of polystyrene spheres were used as casts, and metals were deposited on the spheres by thermal evaporation. When polystyrene casts were interfering with measurements, degradation of organic substances was performed, and cup-shaped metal NP shells can be also obtained. For the identification of fabricated NPs, field emission scanning electron microscopy (FE-SEM) was used. Both diameters and elements of NPs were identified with observations of secondary electron (SE) and backscattered electron (BE) in the FE-SEM.

By using our method, more than 500 types of NPs were fabricated. Metal shell layers were formed by thermal evaporation; therefore, multi-layered NPs can be easily fabricated with sequential evaporation. We used double-layered NPs; outer is Au for easy immobilization of biomolecules to use these NPs as labels of biomarkers, and inner layer is various to apply label varieties. Spatial distributions and diameters of NP labels were identified by SE observation, and NP elements were identified by BE observation as the difference of intensities in the image caused by the difference of atomic number of inner metal. We call it ''adaptive SEM'' technology (i.e., NP identification is "adaptive" for various samples). These results indicate a possibility for quantitative in situ detection of expressed biomarkers in a cell by the suggested technology.

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Single Cell Microbioreactor for observing Dynamic Gene Expression and Cell Response to Environment in Bacteria

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Biological systems have been shown to implement a low-pass filter in order to distinguish high frequency noise from a lower frequency input signal, which is essential to a cellular system to adapt or sustain fitness in fluctuating environment conditions. Gene expression has been shown to exhibit noise leading to phenotypic heterogeneity of a cell population. Many microfluidic techniques have been developed in order to study single cell inducible gene expression, however rely solely on diffusion timescales to effectively alternate between inducer concentrations. In our approach, we developed a microfluidic platform for single cell analysis that allows for dynamic control of a target cell in oscillating well-defined culture media and inducer concentrations. The hydrodynamic trap enables confinement and manipulation of single cells in free solution using the sole action of fluid flow. Automated feedback control is integrated into the device using an ''on-chip'' valve, which allows for precise confinement of cells in free solution. During observation, cells are confined at a fluid stagnation point generated by planar extensional flow in a cross-slot microfluidic geometry, thereby enabling non-perturbative trapping of cells for long time scales. Using this platform, we investigated the effect of small molecule inducers on gene expression in the lac operon using fluorescent reporter proteins and cell growth rates as a proxy of cellular fitness. We applied our technique to determine the cut-off frequency associated with periodic stimuli for the lac circuit in E. coli, wherein the cut-off frequency in the case of the low-pass filter is the higher frequency when response begins to attenuate. We observed that single cell gene expression depends on the correlation between growth rate and frequency of exposure to inducer concentrations.

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A Microfluidic Platform for Controlled Ligand Exposure and Visualization of Cellular Response

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We report here a microfluidic device with a unique architecture for capturing and isolating single cells, as well as providing strict control of reagent delivery to the cells. The device contains individualized cell traps with multiple inlet channels to allow flow-focusing of reagents for controlled ligand-receptor interaction studies. In this configuration, a change in relative inlet pressures causes immediate change in the relative widths of the reagent streams which allows timed exposure of cells to ligands of interest. We demonstrate the capabilities of this device by imaging cellular calcium response as a function of ligand pulse width, inter-pulse interval, and frequency. Rat basophilic leukemia (RBL) cells that endogenously express the IgE receptor are loaded with Fluo-4, a calcium indicator dye, and then captured in the device traps. Cells were systematically exposed to various ligand (multivalent crosslinker DNP-BSA) pulse widths (5 seconds to 2 minutes) and calcium response monitored with high-resolution fluorescence imaging. A significant delay between initial ligand exposure and calcium mobilization was observed. The relationship between pulse width, inter-pulse interval, and frequency and the temporal profile of the calcium response is currently under investigation.

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Accelerating the Development of Hippocampal Neurons using Nanopillar Structures

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During the development of a neuron cell, multiple dendrites and a single axon, which have molecularly and functionally distinct domains, will generate from the soma to enable the directional communication between cells. The initiation and extension of such structures are critical to neuron development and neural circuit formation. In vitro neuron culture system has been a major model to study axon initiation and elongation, and many regulating genes have been identified in the in vitro model. However, only a few of the genes have been proven to be required in vivo, which may mainly due to the lack of extracellular cues in the in vitro model. The importance of extracellular cues to axon initiation and outgrowth is therefore emerging as a major theme in neural development. Nanostructures and nanomaterials serve as promising candidates to provide topographical cues to neuronal adhesion and development. Previous studies showed that neuron cells were able to sense nanoscale structures and responded differently in their neurite extension. In the present work, we use patterned nanopillar structures as controllable topographical cues to culture hippocampal neurons, and found that nanopillars have significant guidance effect on neurite outgrowth and elongation. More interestingly, the axon specification occurs in the first 12 hours after cell plating, which is much earlier than the usual time point for cells growing on normal flat surfaces. It indicated that the topographical cues can indeed accelerating neural development. We further studied this topographical influence on axon initiation and elongation by varying the diameter, height, pitch and shape of the nanopillars, and different effects were observed. This work will provide new insights on the role of topographical cues for neuronal development in vivo, as well as the possibility of using nanoscale topographic features to control neuronal development.